REMARKS

Claims 53-58, 71-73 and 86-95 are pending. A Final Office Action was mailed on September 1, 2011, with respect to the above-referenced application. Applicants have considered each of the rejections raised in the Final Office Action and offer the following amendments and remarks in response. Reconsideration of the rejections is respectfully requested.

By the present amendment, claims 53-55, 91 and 94 are amended, and new claims 96 to 104 are added.

Claims 53-55 have been amended to delete recitation of an immunogenic fragment or variant of the claimed polypeptide and of a cell culture supernatant. Claims 91 and 94 have been amended with respect to antecedent basis. Support for new claims 96-98 may be found in previously pending claims 53-55 and in the specification at, for example, the paragraph spanning pages 18 and 19 of the PCT specification. Support for new claims 99-101 may be found in the specification at, for example, page 6, lines 20-22, and page 12, lines 11-14. Support for new claims 102-104 may be found in the specification at, for example, page 10, lines 20-28.

No new matter has been added by the amendments.

Election/Restrictions

Further to the Examiner's remarks in connection with the request for rejoinder of SEQ ID NOs: 22 and 23, new claims 96-98 have been added to recite these sequence identifiers.

Rejection Under 35 U.S.C. § 112

I. Claims 53-58, 71-73 and 86-95 are rejected under 35 U.S.C. 112, first paragraph, as allegedly failing to comply with the written description requirement. More specifically, the Examiner alleges that the specification describes species with 83% (*C. rodentium* NleA, SEQ ID NO: 22) and 89.5% (EPEC NleA, SEQ ID NO: 23) sequence identity to the claimed SEQ ID NO:

24 (EHEC NleA), but allegedly does not demonstrate possession of the full genus of fragments or variants of a polypeptide at least 75% identical to SEQ ID NO: 24, or proteins which have at least 75% sequence identity to SEQ ID NO: 24, that have the function of eliciting an immune response, treating or preventing infection in an animal and reducing colonization or shedding of any A/E pathogen.

Claims 53-55, which are the only pending independent claims, have been amended without prejudice or disclaimer and without acquiescence to the Examiner's assertions, to recite that the claimed polypeptide comprises an amino acid sequence having at least 81% sequence identity to SEQ ID NO: 24. Support for this amendment may be found in the specification at for example Figure 11C, which shows multiple protein sequence alignments of NleA polypeptides from different organisms, and further demonstrated in Appendix A, enclosed herewith, which compare SEQ ID NO: 24 to *C. rodentium* NleA (SEQ ID NO: 22, 81% identity) and EPEC NleA (SEQ ID NO: 23, 89% identity).

Claims 53-55 have been amended without prejudice or disclaimer and without acquiescence to the Examiner's assertions, to cancel the term "immunogenic fragment or variant." To the extent that the term "immunogenic fragment" appears in new claims 96-98, the Applicants respectfully submit that, even for the written description requirement, specific examples are not necessary as long as the skilled person would recognize that the Applicants were in possession of the claimed invention. For example, it has been held that proper disclosure of an antigen provides an adequate written description of an antibody claimed by its binding affinity to that antigen (see the Manual of Patent Examining Procedure, § 2163, citing *Noelle v. Lederman*, 355 F.3d 1343, 1349, 69 USPQ2d 1508, 1514 (Fed. Cir. 2004)). In the present case, the Applicants respectfully submit that, given the teachings of, for example, the paragraph spanning page 18, line 26, to page 19, line 6, of the instant application, the skilled person would readily recognize that the Applicants were in possession of the claimed polypeptides and immunogenic fragments of at least 10 amino acids thereof, as is claimed.

Rejection Under 35 U.S.C. § 102

Claims 53-58, 71-72 and 86-95 remain rejected under 35 U.S.C. 102(b) as allegedly anticipated by Finlay *et al.* (WO 02/053181) as evidenced by Hideo *et al.* (JP20023550742A2, partial translation attached as Appendix B of the Office Action).

More specifically, the Examiner alleges that Finlay *et al.* teach methods for eliciting an immune response against an A/E pathogen or component thereof, or for reducing colonization of an A/E pathogen, or of reducing shedding (thus allegedly treating an infection by an A/E pathogen) in an animal by administering an effective amount of a composition comprising a culture supernatant, and that the culture supernatant of Finlay *et al.* is prepared from *E. coli* EHEC O157:H7 under identical conditions as SEQ ID NO: 24 of the instant specification. The Examiner further alleges that Hideo *et al.* teach that *E. coli* EHEC O157:H7 makes a protein comprising the sequence of SEQ ID NO: 24.

The Examiner therefore concludes that the culture supernatant of Finlay *et al.* is a composition or culture supernatant which comprises a polypeptide which comprises an amino acid sequence substantially identical to the sequence of SEQ ID NO: 24 and inherently comprises 20% of the cell protein present in the composition. The Applicants thank the Examiner for the explanation of "inherently comprises 20% of the cell protein present in the composition" in the context of this rejection and assume that reference is being made to the cell culture supernatant of Finlay *et al.*, rather than Hideo *et al.*, since the latter does not disclose a cell culture supernatant.

Solely to expedite prosecution, and without acquiescence to the Examiner's rejections, claims 53-55 have been amended without prejudice or disclaimer to delete recitation of a cell culture supernatant. The Applicants reserve the right to pursue cancelled subject matter in a continuation or other application. Accordingly, this rejection should be withdrawn.

Rejections Under 35 U.S.C. § 103

Claims 53-58, 71-72, 86 and 89-95 are rejected under 35 U.S.C. 103(a) as allegedly obvious over Hideo *et al.* (*supra*) in view of Li *et al.* (US2004/0180060).

More specifically, the Examiner alleges that Hideo *et al.* disclose a method of eliciting an immune response against *E. coli* O157:H7 by administering an effective amount of a composition for inducing an immune response against *E. coli* O157:H7 comprising a protein having at least 75% identity to SEQ ID NO: 24. The Examiner further alleges that Hideo *et al.* also disclose treating an infection by *E. coli* O157:H7 using the composition and that treatment of the *E. coli* infection will result in reduction in colonization and shedding of *E. coli* in an animal. With respect to Li *et al.*, the Examiner alleges that this reference teaches that "*E. coli* O157:H7 colonizes the intestines or ruminants and other animals and generally does not cause overt disease" and that "shedding of the *E. coli* O157:H7 into feces of colonized animals serves as a source of *E. coli* infection in humans and it is important therefore to eradicate or reduce O157:H7 shedding in animals particularly cattle to prevent human infection." The Examiner further alleges that Li *et al.* teach "adjuvanted vaccines comprising O157:H7 antigens for the reduction of O157:H7 colonization in animals or ruminants particularly cattle and methods of administering same to cattle to prevent shedding."

While the Examiner concedes that Hideo *et al.* do not disclose practicing the method with a ruminant, including a bovine or ovine, and do not disclose that the composition comprises an adjuvant, the Examiner alleges that "it would have been *prima facie* obvious to one of ordinary skill in the art at the time that the instant invention was made to have practiced the method of Hideo *et al.* in ruminants or cattle thus resulting in the instant invention with a reasonable expectation of success. The Examiner finds motivation to do so in the teachings of Li *et al.* that "the shedding of *E. coli* O157:H7 into feces of colonized animals serves as a source of *E. coli* infection in humans and it is important therefore to eradicate or reduce O157:H7 shedding in animals particularly cattle to prevent human infection. The Examiner further alleges that "it

would have been *prima facie* obvious to one of ordinary skill in the art at the time that the instant invention was made to have included an adjuvant in the composition for practicing said method in ruminants or cattle, as Li *et al.* teach that adjuvants can be combined with O157:H7 antigens for the reduction of O157:H7 colonization in animals or ruminants particularly cattle and to prevent shedding. The Examiner further alleges that the adjuvant of Li *et al.* is beneficial as it helps to stimulate an immune response in the vaccinated ruminant.

This rejection is respectfully traversed. As indicated herein, claims 53-55 are the only pending independent claims, and therefore these rejections will be addressed with respect to these claims only. The remaining claims at issue under this rejection are dependent claims and by definition subject to the limitations of the independent claims. Claims 53-55 are directed to methods for eliciting an immune response against an A/E pathogen or component thereof, or for reducing colonization or shedding of an A/E pathogen, in an animal by administering an effective amount of a composition including an isolated polypeptide comprising an amino acid sequence having at least 81% sequence identity to the sequence of SEQ ID NO: 24.

Further to the Examination Guidelines for Determining Obviousness Under 35 U.S.C. § 103 in View of the Supreme Court Decision in KSR International Co. v. Teleflex Inc. (72 Fed. Reg. 57,526 (Oct. 10, 2007); hereafter the "Guidelines"), which takes into consideration *Graham v. John Deere Co.* as cited by the Examiner, a proper rejection under 35 U.S.C. § 103 requires:

1. a finding that the prior art included each element claimed, although not necessarily in a single prior art reference, with the only difference between the claimed invention and the prior art being the lack of actual combination of the elements in a single prior art reference;

2. a finding that one of ordinary skill in the art could have combined the elements as claimed by known methods, and that in combination, each element merely would have performed the same function as it did separately;

3. a finding that one of ordinary skill in the art would have recognized that the results of the combination were predictable; and

4. whatever additional findings based on the *Graham* factual inquiries may be necessary, in view of the facts of the case under consideration, to explain a conclusion of obviousness.

The Applicants respectfully submit that the teachings of Hideo *et al.*, alone or in combination with the teachings of Li *et al.*, do not meet these requirements, as discussed below.

Firstly, Hideo *et al.* examine nucleotide sequences from enterohemorrhagic *E. coli* (EHEC) O157:H7 SAKAI and assert that approximately two thousand (2000) sequences (referred to hereafter as the "EHEC-specific sequences") are not present in non-pathogenic *E. coli* K-12[1]. Based on this assertion, Hideo *et al.* conclude that the EHEC-specific sequences are pathogenic, simply because EHEC O157:H7 is highly pathogenic and *E. coli* K-12 is not.

This conclusion is incorrect. In a study similar to that conducted by Hideo *et al.*, in which EHEC O157:H7 EDL933 was compared to *E. coli* K-12 laboratory strain MG1655, the researchers found 1,387 unique genes (designated "O-islands") and noted the existence of O-islands with "no obvious association with pathogenicity."[2] Perna *et al.* further noted that "[o]nly the locus of enterocyte effacement (LEE) and two of the lambdoid phages … have as yet been experimentally associated with virulence in animal models."[3] It is understood by those of skill in the art that non-pathogenic K12 and EHEC O157:H7 share about 80% sequence identity and are

---

1. See page 40, paragraph [0010], and page 71, paragraph [0014], of the English translation of Hideo *et al.*, hereafter the "Hideo translation," submitted with Applicant's Reply filed April 11, 2011.

2. See Perna *et al.*, Nature 409:529-533, 2001, cited in IDS dated May 19, 2009), at p. 531, col. 1, first paragraph, emphasis added.

3 See Perna *et al.*, Nature 409:529-533, 2001, cited in IDS dated May 19, 2009), at p. 531, col. 1, third paragraph.

about 20% different. Given that the genomes of these organisms are about 4 million base pairs, the difference computes to about 800,000 base pairs. At the time of filing, therefore, the known virulence factors made up only a small fraction of genomic differences between non-pathogenic K12 and pathogenic EHEC O157:H7 (*e.g.,* the LEE region, which encodes the Type III secretion system is 34,000 base pairs, the Shiga toxin is 1,000 base pairs, *etc.*). Accordingly, one of ordinary skill in the art would recognize that not all the EHEC-specific sequences set out in Hideo *et al.* encode virulence factors and furthermore that Hideo *et al.* do not provide any guidance as to which of the large number of EHEC-specific sequences would function as virulence factors and therefore be useful as presently claimed. In this regard, the Applicant notes that " virulence factors" are understood to be molecules required to cause disease, that are not normally required for viability of a micro-organism in non-disease settings. As such, virulence factors are considered useful in eliciting an immune response against the micro-organism.

Secondly, Hideo *et al.* teach predicted amino acid sequences based on the identified EHEC-specific nucleotide sequences and comparison of these predicted amino acid sequences to known sequences from various public databases, using known algorithms[4], as follows:

1) Proteins having unknown function etc.,

2) Proteins which have unknown function, but have significant homology to that of other bacteria,

3) Proteins comprising Insertion Sequences (IS),

4) Proteins derived from phage,

5) Regulatory elements,

6) Proteins relating to fimbriae,

7) Proteins relating to transportation of substance,

8) Proteins relating to synthesis of lipopolysaccharide,

---

4. See page 71, paragraph [0016] of the Hideo translation, submitted in the response to Office Action dated April 11, 2011. Hideo *et al.* classify the predicted amino acid sequences into twelve (12) groups (see pages 71-72, paragraph [0017] of the Hideo translation).

9) Proteins relating to metabolism,

10) Proteins processing DNA/RNA,

11) Proteins relating to pathogenicity, and

12) Other proteins.

SEQ ID NO: 393 of Hideo *et al.*, which is asserted to be identical to SEQ ID NO: 24 (EHEC NleA) of the instant application, is called out in Group 2 (Proteins which have unknown function, but have significant homology to that of other bacteria) and is described as follows at page 154 of the Hideo translation:

> SEQ ID NO: 393 -0.239229, 442, a minor capsid protein precursor, similar to minor capsid protein precursors for example, GpC [Bacteriophage lambda] gi|137565|sp|P03711|VCAC#LAMBD (97% identity in 439 amino acids), capsid assembly protein containing Nu3-homolog;

Capsid proteins are bacteriophage (bacterial virus) proteins used as part of the viral assembly process, and present in the viral coat upon maturation. Tellingly, Hideo *et al.* do not point to SEQ ID NO: 393 as relating to pathogenicity; rather, such sequences are listed in Group 11. By contrast, the Applicants were the first to identify NleA polypeptide (SEQ ID NOs: 22-24) as a virulence factor. The Applicants respectfully submit that, once identified, a virulence factor is recognized to be useful to induce an immune response in animals but prior to such identification there would be no reason to conclude that any EHEC-specific sequence, as taught by Hideo *et al.* would be useful in the methods claimed in the instant application. Rather, Hideo *et al.* teach away from the claimed invention, as a bacteriophage capsid protein would not be expected to be effective in the methods as claimed and the identification of SEQ ID NO: 393 as such would not lead a skilled person to select SEQ ID NO: 393 out of the approximate two thousand EHEC-specific sequences of Hideo *et al.* for use as presently claimed.

Thirdly, Hideo *et al.* merely speculate that some of the EHEC-specific sequences may be useful for various purposes, such as diagnosis, therapy, *etc.*, stating that a protein that is "predicted to be a cell surface protein … may be useful for production of an antibody, vaccine composition, diagnosis of O-157 infection …," that "… there is a possibility that they include a

protein which has an important function in O-157, for example, transportation and metabolism of a substance, processing of nucleic acids, and relates to a regulatory element and pathogenicity..." (see pages 267-270, paragraph [0031] of the Hideo translation, emphasis added). Hideo *et al.* do not provide any experimental data or other evidence in support. The Applicant respectfully submits that this speculation would not lead a skilled person to the claimed invention – at best, the statements of Hideo *et al.* rise to the level of an invitation to experiment.

Finally, Hideo *et al.* make it clear that the contemplated use of the disclosed sequences is in the context of treating infection in a patient:

> O-157 specific nucleic-acid molecule of the present invention, a gene included in it, peptide and nucleic-acid sequence encoded by the gene are useful for diagnosis and/or therapy of O-157 infection and prevention of symptom occurred by the infection. (see page 283, paragraph [0047] of the Hideo translation, emphasis added)
>
> ...
>
> the scope of the present invention includes a vaccine composition including genes and/or polynucleotides of the present invention, and a method for prevention and/or therapy of O-157 infection and symptom occurred by the infection. (see page 283, paragraph [0048] of the Hideo translation, emphasis added)
>
> ...
>
> The present invention relates to a method of reducing the risk of O-157 infection in patients or a method for therapy [of the infection]. This method comprises administration of the vaccine formulation of the present invention to a patient so as to reduce the risk of O-157 infection or provide therapy of infection.(see page 285, paragraph [0053] of the Hideo translation, emphasis added).

The term "infection" is defined as "[i]nvasion by and multiplication of pathogenic microorganisms in a bodily part or tissue, which may produce subsequent tissue injury and progress to overt disease through a variety of cellular or toxic mechanisms" or the "pathological state resulting from having been infected." (see, infection, Dictionary.com, *The American Heritage® Stedman's Medical Dictionary*. Houghton Mifflin Company. http://dictionary.reference.com/browse/infection, accessed: November 18, 2010). Therefore, the term "infection" contemplates that the infected subject or animal exhibits symptoms of clinical disease. By contrast, ruminants may be colonized by and shed highly virulent A/E pathogens, or exhibit an immune response against an A/E pathogen or component thereof, without ever

exhibiting symptoms of overt disease, as is acknowledged by the Examiner.

The Applicant agrees with Li *et al.* that vaccination of animals such as cattle to reduce shedding of *E. coli* O157:H7 is desirable to achieve the downstream goal of preventing human infection. The teachings of Li *et al.* however are directed to vaccine compositions comprising an immunologically active component selected from inactivated or killed whole or subunit *E. coli* O157:H7 antigens in combination with a metabolizable oil and aluminum hydroxide adjuvant. The only reference to any antigen other than inactivated or killed whole bacteria in Li *et al.* is to O-specific polysaccharide (see para. [0017] of Li *et al.*), which would be present on the surface of the bacteria. By contrast, the claimed invention is directed to the use of a secreted virulence factor represented by NleA polypeptide (SEQ ID NOs: 22-24).

The Applicants respectfully submit that using inactivated or killed whole bacteria, or an antigen present on the surface of such bacteria, would not lead to the claimed invention and that Li *et al.* therefore teach away from the claimed invention.

In summary, Hideo *et al.* teach that:

1. Approximately two thousand (2000) nucleotide sequences from enterohemorrhagic *E. coli* O157:H7 SAKAI are not present in non-pathogenic *E. coli* K12 and therefore the EHEC-specific sequences are pathogenic, because EHEC O157:H7 is highly pathogenic and *E. coli* K12 is not;

2. SEQ ID NO: 393, which is asserted to be identical to SEQ ID NO: 24 (EHEC NleA) of the instant application, is of unknown function but has significant homology to a capsid protein, and is not considered to be related to pathogenicity;

3. There is a possibility that the predicted EHEC-specific protein sequences may include a protein that has an important function in O-157; and

4. The EHEC-specific sequences may be useful in treating infection in a patient.

Li *et al*. teach that:

5. Vaccination of animals such as cattle to reduce shedding of *E. coli* O157:H7 is desirable to achieve the downstream goal of preventing human infection; and

6. This goal may be achieved using vaccine compositions comprising an immunologically active component selected from inactivated or killed whole or subunit *E. coli* O157:H7 antigens (*i.e*., O-specific polysaccharide) in combination with a metabolizable oil and aluminum hydroxide adjuvant.

In the present case, the Applicants respectfully submit that, given these teachings, one of ordinary skill in the art would not have recognized that the results of the combination of Hideo *et al.* and Li *et al*. were predictable or would lead to the claimed invention.

The case law clearly holds that an invention "composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *KSR International Co. v. Teleflex Inc*. (550 U.S. 398, 418 (2007)). Rather, "it can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements the way the claimed new invention does." *Id.,* emphasis added. Furthermore, the skilled person must have " perceived a reasonable expectation of success in making the invention in light of the prior art." *Amgen Inc. v. F. Hoffman-La Roche Ltd.*, 580 F.3d 1340, 1362 (Fed. Cir. 2009). However, the Examiner has given no explanation or reasoning as to why a skilled person would select SEQ ID NO: 393 out of the over two thousand sequences presented by Hideo *et al*., as useful to elicit an immune response in a ruminant or to reduced colonization or shedding, particularly in view of teachings that this polypeptide did not relate to pathogenicity. Certainly the general teachings of Li *et al*. that vaccination of animals is useful, together with specific teachings of an entirely different approach to that claimed in the present invention, do not in any way point to the identification of NleA as a virulence factor. To state otherwise is hindsight reconstruction, which is impermissible.

Therefore, Hideo *et al.*, considered alone or in combination with Li *et al*., do not render

the claimed invention obvious and this rejection should be withdrawn.

Claim 88 is rejected under 35 U .S.C. 103(a) as allegedly obvious over Hideo *et al.* (*supra*) as evidenced by Li *et al.* (*supra*) in view of Finlay *et al.* (*supra*).

More specifically, the Office Action alleges that Hideo *et al.* disclose a method of eliciting an immune response against *E. coli* O157:H7 by administering an effective amount of a composition for inducing an immune response against *E. coli* O157:H7 comprising a protein identical to SEQ ID NO: 24. The Office Action further alleges that Hideo *et al.* also disclose treating an infection by *E. coli* O157:H7 using the composition. The Office Action further alleges that treatment of the *E. coli* infection will result in reduction in colonization and shedding of *E. coli* in an animal. The Office Action further alleges that it is inherent that the methods of Hideo *et al.* are to be practiced in animals since Wright *et al.* teach that *E. coli* or *E. coli* O157:H7 infect animals.

While the Office Action concedes that Hideo *et al.* do not disclose that the composition further comprises EspA, EspB, EspD, EspC, intimin and Tir or an adjuvant, the Office Action alleges that Finlay *et al.* teach methods for eliciting an immune response against an A/E pathogen or component thereof, or for reducing colonization of an A/E pathogen, or of reducing shedding (thus allegedly treating an infection by an A/E pathogen) in an animal by administering an effective amount of a composition comprising a culture supernatant where the composition includes EspA, EspB, EspD, EspC, intimin and Tir and/or further includes an adjuvant. The Office Action further alleges that Finlay *et al.* teach that the composition treats the EHEC infection and/or reduces colonization of the animal and teach that administration of the composition to an animal stimulates an immune response against one or more secreted antigens, such as EspA and Tir, which blocks attachment of the EHEC to intestinal epithelial cells.

The Office Action therefore alleges that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the instant invention was made to have combined the composition of Hideo *et al.* with that of Finlay *et al.*, thus resulting in the instant method

(wherein the composition further comprises EspA, EspB, EspD, EspC, intimin and Tir or further comprises an adjuvant) with a reasonable expectation of success. The Office Action finds the motivation to do so because both compositions are allegedly individually taught in the prior art to be useful for the same purpose *i.e.*, inducing an immune response against *E. coli* EHEC O157:H7 and Finlay *et al.* allegedly provide additional motivation in that administration of the composition to an animal stimulates an immune response against one or more secreted antigens, such as EspA and Tir, that blocks attachment of the EHEC to intestinal epithelial cells.

This rejection is respectfully traversed. As discussed herein, in the sections addressing the rejections under 35 U.S.C. 102(b) and 103(a) with respect to Hideo *et al.*, one of ordinary skill in the art would not have recognized that the results of the combination of Hideo *et al.* and Wright *et al.*, with or without Finlay *et al.*, were predictable since Hideo *et al.* provide no guidance as to which of approximately two thousand (2000) sequences may be useful and Wright *et al.* do not cure this defect. The addition of Finlay *et al.* also do not cure the defects in Hideo *et al.* and Wright *et al.* More specifically, as indicated herein, the inventors of the present application were the first to identify NleA polypeptide (SEQ ID NOs: 22-24) as a virulence factor, which would then lead a skilled person to conclude that it would be useful to induce an immune response. Prior to such identification there would be no reason to conclude that any protein would be useful in the methods claimed in the instant application and Finlay *et al.* do not provide such identification. Accordingly Hideo *et al.*, considered alone or in combination with Wright *et al.* and/or Finlay *et al.*, do not render the claimed invention obvious.

Conclusion

The Applicant respectfully requests that a timely Notice of Allowance be issued in this case.

Respectfully submitted,

Date: February 1, 2012 /Gene H. Yee/

Gene H. Yee, Reg. No. 57,471

Fenwick & West LLP
801 California Street
Mountain View, CA 94041
Telephone: (206) 389-4535
Facsimile: (650) 398-5200

APPENDIX A
Homologies and comparisons of NleA amino acid sequences

1. Sequence alignment using BlastP

A. EHEC NleA vs. *Citrobacter rodentium* (CR) NleA: 81% identity

```
        Identities = 359/443 (81%), Positives = 391/443 (88%), Gaps = 15/443 (3%)

EHEC NleA  1    MNIQPTIQSGITSQNNQ-HHQTEQIP-STQIPQSELPLGCQAGFVVNIPDDIQQHAPECG  58
               MNIQP I SGIT+QNNQ HH  EQ+P S+ IP+S+LP  C+AGFVV+IP+DIQQH PECG
CR NleA    1    MNIQPNIHSGITTQNNQQHHHAEQVPVSSSIPRSDLPPNCEAGFVVHIPEDIQQHVPECG  60

EHEC NleA  59   ETTALLSLIKDKGLLSGLDEYIAPHLEEGSIGKKTLDMFGLFNVTQMALEIPSSVSGISG  118
               ETTALLSLIKD+GLLSGLD+Y+APHLEEGS+GKK LD FGLFNVTQMALEIPSSV GISG
CR NleA    61   ETTALLSLIKDEGLLSGLDKYLAPHLEEGSLGKKALDTFGLFNVTQMALEIPSSVPGISG  120

EHEC NleA  119  KYGVQLNIVKPDIHPTSGNYFLQIFPLHDEIGFNFKDLPGPLKNALSNSNISTTAVSTIA  178
               KYGVQ+NIVKPDIHPT+GNYFLQ+FPLHDEIGFNFKDLPGPLKNAL+NS+IS TA
CR NleA    121  KYGVQMNIVKPDIHPTTGNYFLQLFPLHDEIGFNFKDLPGPLKNALTNSSISATA-----  175

EHEC NleA  179  STGTSATTSTVTTEPKDPIPWFGLTAQVVRNHGVELPIVKTENGWKLVGETPLTPDGPKA  238
                        STV   P DP+PWFGLTAQVVRNHGVELPIVKTENGWKLVGETPLTPDGPKA
CR NleA    176  --------STVAPTPNDPMPWFGLTAQVVRNHGVELPIVKTENGWKLVGETPLTPDGPKA  227

EHEC NleA  239  NYTEEWVIRPGEADFKYGASPLQATLGLEFGAHFKWDLDNPNTKYAVLTNAAANALGALG  298
               NYTEEWVIRPGEADFKYG SPLQATLGLEFGAHFKWDLDNPNTKYA+LTNAAANA+GA G
CR NleA    228  NYTEEWVIRPGEADFKYGTSPLQATLGLEFGAHFKWDLDNPNTKYAILTNAAANAIGAAG  287

EHEC NleA  299  GFAVSRFASTDPMLSPHIGAMVGQAAGHAIQYNTPGLKPDTILWWAGATLGAADLNKAEF  358
               GFAVS+    DPMLSPH+GAM+GQAAGHA+Q NTPGLKPDTILWWAGAT GAADLNKAEF
CR NleA    288  GFAVSKVPGIDPMLSPHVGAMLGQAAGHAVQCNTPGLKPDTILWWAGATFGAADLNKAEF  347

EHEC NleA  359  EVARFTDYPRIWWHAREGAIFPNKADIEHATGADIRAMEEGIPVGQRHPNPEDVVIDIES  418
               +  RFTDYPRIW+HAREGA+FPNK DI   TGADI+AMEEG+PVG +HP PEDVVIDIE
CR NleA    348  DKVRFTDYPRIWFHAREGALFPNKQDIARVTGADIKAMEEGVPVGHQHPKPEDVVIDIEG  407

EHEC NleA  419  NGLPHHNPSNHVDIFDIIQETRV  441
                  PHHNPSN+VD F+IIQETRV
CR NleA    408  GNSPHHNPSNYVDTFEIIQETRV  430
```

B. EHEC NleA vs. EPEC NleA: 89% identity

```
        Identities = 393/441 (89%), Positives = 410/441 (93%), Gaps = 1/441 (0%)

EHEC NleA  1    MNIQPTIQSGITSQNNQHHQTEQIPSTQIPQSELPLGCQAGFVVNIPDDIQQHAPECGET  60
               MNIQP + SGIT+QNN+HH  EQ   TQIPQSELP GC+ GFVV+IP+D+Q+HAPECGET
EPEC NleA  1    MNIQPIVTSGITTQNNRHHHAEQTSPTQIPQSELPNGCETGFVVHIPEDMQRHAPECGET  60

EHEC NleA  61   TALLSLIKDKGLLSGLDEYIAPHLEEGSIGKKTLDMFGLFNVTQMALEIPSSVSGISGKY  120
               TALLSLIKD+GLLSGLD+Y+APHLEEGS GKK LDMFGLFNV+QMALEIPS+V GISGKY
EPEC NleA  61   TALLSLIKDEGLLSGLDKYLAPHLEEGSAGKKALDMFGLFNVSQMALEIPSTVPGISGKY  120

EHEC NleA  121  GVQLNIVKPDIHPTSGNYFLQIFPLHDEIGFNFKDLPGPLKNALSNSNISTTAVSTIAST  180
               GVQLNIVKPDIHPTSGNYFLQIFPLHDEIG NFKDLPGPLKNALSNSNI TT VST AST
EPEC NleA  121  GVQLNIVKPDIHPTSGNYFLQIFPLHDEIGINFKDLPGPLKNALSNSNIPTT-VSTAAST  179

EHEC NleA  181  GTSATTSTVTTEPKDPIPWFGLTAQVVRNHGVELPIVKTENGWKLVGETPLTPDGPKANY  240
                 SATTSTVTT  KDPIPWFGLTAQVVRNHGVELPIVKTENGWKLVGETPLTPDGPKANY
EPEC NleA  180  IASATTSTVTTASKDPIPWFGLTAQVVRNHGVELPIVKTENGWKLVGETPLTPDGPKANY  239

EHEC NleA  241  TEEWVIRPGEADFKYGASPLQATLGLEFGAHFKWDLDNPNTKYAVLTNAAANALGALGGF  300
               TEEWVIRPGEADFKYGASPLQATLGLEFGAHFKWDLDNPNTKYAVLTNAAANALGA+GGF
```

```
EPEC NleA  240  TEEWVIRPGEADFKYGASPLQATLGLEFGAHFKWDLDNPNTKYAVLTNAAANALGAVGGF  299

EHEC NleA  301  AVSRFASTDPMLSPHIGAMVGQAAGHAIQYNTPGLKPDTILWWAGATLGAADLNKAEFEV  360
                AVSRF  TDPMLSPHIGAMVGQAAGHAIQYNTPGLKPDTILWWAG TLG ADLNKAEF
EPEC NleA  300  AVSRFTGTDPMLSPHIGAMVGQAAGHAIQYNTPGLKPDTILWWAGTTLGLADLNKAEFGE  359

EHEC NleA  361  ARFTDYPRIWWHAREGAIFPNKADIEHATGADIRAMEEGIPVGQRHPNPEDVVIDIESNG  420
                ARFTDYPRIWWHAREGAIFPNKADIEHATGADIRAMEEG+ VGQRHPNPEDVVI+IESN
EPEC NleA  360  ARFTDYPRIWWHAREGAIFPNKADIEHATGADIRAMEEGVSVGQRHPNPEDVVINIESNN  419

EHEC NleA  421  LPHHNPSNHVDIFDIIQETRV  441
                 PHHNPSN+VD  DIIQETRV
EPEC NleA  420  SPHHNPSNYVDTVDIIQETRV  440
```